an integer greater than or equal to 0, and t is an integer greater than or equal to 8 such that

T0740

$$\begin{matrix} -(N)_t \\ -(N')_t \end{matrix}$$

forms a double stranded moiety selected from a minimally cross-hybridizing set of oligonucleotides such that each oligonucleotide of the set differs from every other oligonucleotide of the set by at least two basepairs.

A6

28. (Amended) The composition of claim 27 wherein r is between 0 and 12, inclusive, t is an [~~interger~~] integer between 8 and 24, inclusive, and z is a phosphate group[~~, and said double stranded moiety~~

$$\begin{matrix} -(N)_t \\ -(N')_t \end{matrix}$$

~~is a member of a minimally cross-hybridizing set~~].

REMARKS

Claim 1, 15, 17, 19, 24, 25, 27 and 28 have been amended. Claims 1-29 are currently pending in the application.

The amendments to claims 1, 15, 17, 19, 24, 25, 27 and 28 clarify: i) the notion that multiple encoded adaptors may be ligated to a single type of polynucleotide, e.g. as illustrated in figures 1a-1e; ii) that encoded adaptors are double stranded DNAs (bases for this amendment are set forth below); iii) that oligonucleotide tags of encoded adaptors are selected from a minimally cross-hybridizing set of oligonucleotides having lengths within the range of 8 to 20 nucleotides (for single stranded tags) or basepairs (for double stranded tags); and iv) that oligonucleotides of a minimally cross-hybridizing set differ from one another by at least two nucleotides. Basis for iii) is on page 19, line 1; and basis for iv) is page 3, lines 30-32.

No new matter has been added by the amendments. Reconsideration is respectfully requested.

Double Patenting Rejections

The Examiner provisionally rejected claims 1-29 under 35 U.S.C. 101 for

A

claiming the same subject matter as claims 1-29 of co-pending patent application Ser. No. 08/862,610.

Applicants direct the Examiner's attention to the copy of the Express Abandonment of U.S. application Ser. No. 08/862,610 attached as Exhibit A. In view of the abandonment of the application, Applicants respectfully request that the Examiner withdraw the above double patenting rejection.

The Examiner rejected claims 1, 2, 6-8, and 11-12 under the doctrine of obviousness-type double patenting with respect to various claims of U.S. patent 5,599,675 ('675). The Examiner points out that the embodiment of claim 49 of '675 calls for a probe that is assembled at the end of a target polynucleotide from two single stranded oligonucleotides that form a duplex containing a type IIs restriction endonuclease recognition site. The Examiner apparently reasons that one of ordinary skill in the art would have been led the instant invention by observing the use of the single stranded oligonucleotides in the embodiment of claim 49, particularly as one of the single strands carries a fluorescent label, as do the exemplary tag complements of the present invention (e.g. page 43, lines 7-12).

Applicants respectfully disagree. First, an important feature of the present invention is the use of "encoded adaptors" that contain an oligonucleotide tag (see claim 1). Exemplary encoded adaptors are shown on pages 42 and 43 as SEQ ID NOs 10 through 25. *The probes of claim 49, which are illustrated in Figure 1f of '675, do not include oligonucleotide tags*. Thus, contrary to the Examiner's assertion, the probes of '675 and the encoded adaptors of the present case do not have the same structure. The presence of an oligonucleotide tag in an encoded adaptor permits the identification of the sequence of nucleotides in the protruding strand of the adaptor after ligation to a polynucleotide. For a four-nucleotide protruding strand, this means that as many as 256 different 4-nucleotide sequences can be identified in each cycle of ligation and cleavage. With the use of fluorescent probes alone (as taught in '675) at best only 4 or 5 sequences could be identified* in each cycle of ligation and cleavage.

Second, not only do the probes of '675 fail to include oligonucleotide tags, but there is no suggestion in '675, either alone or in combination with any references of record, that it would be advantageous to employ such tags

* Bergot et al, U.S. patent 5,366,860 (included with the enclosed Information Disclosure Statement), col. 1-2, explain this limitation of fluorescent dyes.

A

as a means of *identifying* sequences of nucleotides. The single stranded oligonucleotides of the embodiment of Figure 1f of '675 are used to assemble a probe that has a functioning nuclease recognition site, and (optionally) to provide a fluorescent label. At best they function to identify single nucleotides, not sequences, and clearly the concept of minimally cross-hybridizing set is not disclosed or suggested. Thus, one of ordinary skill in the art would not have been led to the present invention by a knowledge of the teachings of '675. Accordingly, Applicants respectfully request that the rejection be withdrawn.

The Examiner rejected claims 15-18 under the doctrine of obviousness-type double patenting with respect to claims 1-3, 11-12, 21, 25, and 27 of U.S. patent 5,604,097 ('097) when considered in view of U.S. patent 5,599,675 ('675). The Examiner averred that the present invention discloses a method of sequencing polynucleotides that comprises the steps of attaching, sampling, sorting, ligating, and identifying. '097 discloses a method of sequencing polynucleotides that comprises the steps of attaching, sorting, and identifying, and '675 discloses the step of ligating. Therefore, the steps of the present invention would have been obvious to one of ordinary skill in the art by combining the teachings of '097 with those of '675, which would supply the ligation step to give the method of the present invention.

Applicant respectfully disagrees with the Examiner's analysis. Again, nothing in either '675 or '097 discloses or suggests the use of *oligonucleotide tags in the probes* that are used in the "identifying" step of the present invention. '097 discloses the use of oligonucleotide tags for *sorting* the target polynucleotides onto solid phase supports, but otherwise '097 does not suggest that such tag may have other uses. In particular, neither reference teaches an appreciation of the problem addressed by the combination of oligonucleotide tags and adaptors; namely, the problem of carrying out enzymatic reactions on solid phase supports, as described on page 2, lines 10-21, of the specification. Therefore, one of ordinary skill in the art would not have found the instant invention obvious over '097 and '675, either alone or in combination, because based on '675 and '097 alone he or she would have no motivation to make the combination. Accordingly, Applicant respectfully request that the rejection be withdrawn.

35 U.S.C. 112 Second Paragraph

The Examiner rejected claim 1-29 under 35 U.S.C. 112 second paragraph because

of lack of clarity with respect to the following terms or phrases: a) "encoded adaptor" and "minimally cross-hybridizing set," b) the implementation of the "ligating" step in claims 1-23, c) the implementation of the "attaching" step in claims 15-18, d) lack of antecedent basis for the phrase "the nucleotide sequences" of claims 1-16 and 19-22, e) lack of antecedent basis for the phrase "said single stranded moiety $(N)_t$" in claim 25 and "said double stranded moiety" in claim 28, and f) lack of antecedent basis for the phrase "the one or more encoded adaptors" in claims 15 and 16.

Applicants respectfully disagree, particularly in view of the above amendments. In regard to a), the term "encoded adaptor" is defined extensively throughout the specification, for example:

i) page 3, lines 4-10;
ii) page 13, lines 3-26;
iii) page 21, line 10, to page 24, line 22; and
iv) page 42, line 19, to page 43, line 4.

In particular, on page 21, lines 17-19, the synthesis of encoded adaptors is described as follows:

> "Typically, after synthesis of complementary strands, the strands are combined to form a double stranded adaptor."

The term "adaptor" as used in molecular biology refers to a short double stranded DNA molecule usually having at least one protruding strand which is typically used to covalently join separate double stranded DNAs (or separate ends of a single large double stranded DNA, e.g. as in "ring closure" reactions) by action of a DNA ligase. This usage is corroborated in the following exemplary references:

1) Maclean, Dictionary of Genetics & Cell Biology, page 7 (New York University Press, 1987, New York)(attached as Exhibit B);
2) Szybalski, Gene 40: 169-173 (1985)(see Fig. 1 and page 171, second column);
3) Sibson, International Application PCT/GB95/00109 (copy enclosed)(see description starting on page 7, line 18);
4) Kato, Nucleic Acids Research, 23: 3685-3690 (1995) and 24: 394-395 (1996)(copies enclosed);
5) Kato, U.S. patent 5,707,807 (copy enclosed)(see claim 1); and

6) Sibson, U.S. patent 5,728,524 (copy enclosed)(see claim 1).

In view of i) the amendment clarifying that an encoded adaptor is a double stranded DNA and ii) the extensive description of encoded adaptors in the specification, Applicants submit that there would be no lack of clarity as to the definition of the term to one of ordinary skill in the art.

As to the term "minimally cross-hybridizing set" in reference to oligonucleotide tags, the above amendments make it clear that every oligonucleotide of a minimally cross-hybridizing set must differ from every other oligonucleotide of the same set by at least two nucleotides. Basis for the amendments is page 3, lines 30-32. Applicants submit that the amendments obviate the Examiner's concern with respect to the definition of the term.

In regard to b)("ligating"), Applicants respectfully disagree that the step needs further clarification. As pointed out--and exemplified--in the specification, the ligation step may be carried out chemically (page 13, lines 3-26) or enzymatically (page 25, line 11, to page 27, line 25; and Examples 1 & 2). Applicants submit that there are several template-driven chemically based ligation methods suitable for use with the invention that would be accessible to one of ordinary skill in the art. In particular, the many cited references on page 13, lines 6-10, of the specification provide an exemplary listing of such approaches. Applicant also respectfully point out that U.S. patents 5,552,278; 5,599,675; 5,714,330) (of record or copies enclosed) all use the term in an identical manner as in the present case. This is strong evidence that those with ordinary skill in the art would not be confused by its usage.

In regard to c)("attaching" in claims 15-18), Applicant respectfully disagrees that the step needs further clarification. Two primary methods for carrying out the attaching step are disclosed in the specification: i) use of primer containing a tag sequence in first strand synthesis (page 29, line 18, to page 33, line 5), and ii) insertion of target polynucleotides into a vector containing a tag repertoire (page 33, lines 17-22; and page 39, line 28, to page 40, line 34). It is not clear from the Examiner's remarks what more would be needed, particularly in view of the fact that the step involves convention molecular biological techniques accessible to anyone with ordinary skill in that field.

In regard to d)(antecedent basis for "the nucleotide sequence in claims 1 and 19), this has been corrected by the above amendments.

In regard to e)(antecedent basis of phrase "said single stranded moiety $(N)_t$"), this has been corrected by the above amendments.

In regard to f)(antecedent basis of the phrase "the one or more encoded adaptors"

in claim 15, page 64, line 30), Applicants direct the Examiner's attention to page 64, line 24, where she will find the antecedent basis.

In view of the above comments and amendments, Applicants submit that the application fulfills all the requirements of 35 U.S.C. 112 second paragraph and respectfully requests that all rejections thereunder be withdrawn.

35 U.S.C. 103

In part 8. or the Office Action, the Examiner rejected claims 1-10, 12-14, and 19-23 under 35 U.S.C. 103(a) as being unpatentable over Brenner, U.S. patent 5,599,675 ('675) for essentially the same reasons as summarized above in the obviousness-type double patenting rejection based on '675.

Applicants respectfully disagree with the Examiner's analysis for the reasons set forth above. Furthermore, as to the Examiner's statement that "one having ordinary skill in the art would have been motivated to use an encoded adaptor in the method of Brenner to determine a nucleic acid sequence because it would have been expected to serve the same purpose as the probe of Brenner," Applicants especially disagree. The Examiner must state specifically where in '675 there is a suggestion that would lead one skilled in the art i) to recognize or appreciate the technical problem addressed by encoded adaptors, ii) to determine that such a problem could be solved by combining oligonucleotide tags with adaptors, or iii) to recognize any other reason for combining oligonucleotide tags and adaptors. Applicant submit that without such reasoning the Examiner has merely identified the components of Applicants' invention in the cited references and *by hindsight* has deemed it obvious to combine them. 35 U.S.C. 103(a) requires more.

Applicants further submit that the fact that the tag complements serve the same purpose--i.e. delivering a fluorescent label, as accomplished by the probes of Figs. 1f and 1g of '675--has little relevance to the obviousness determination without some additional concrete suggestion that the use of such probes would have led one with ordinary skill to use the encoded adaptors as defined in the claims. Applicants submit that the Examiner's focus on the embodiment of Figs. 1f and 1g of '675 is misplaced. The focus should be on the importance of the oligonucleotide tags as a component of the encoded adaptors. Applicants respectfully request that the rejection be withdrawn.

In part 9. of the Office Action, the Examiner rejected claims 11 and 15-16 under 35 U.S.C. 103(a) as being unpatentable over Brenner ('675) in view of Brenner ('097) for essentially the same reasons as summarized above in the obviousness-type double

patenting rejection based on '097 in view of '675.

Applicant respectfully disagrees with the rejection for the reasons set forth above. Namely, the Examiner has merely identified the elements of the invention in the cited references, but has not provided any reasoning based on the disclosures for why one with ordinary skill in the art would be led to combine the elements to arrive at Applicants' invention. Moreover, Applicants disagree that the excerpt of '097 in column 5, lines 51-56 discloses "the formation of the Hoogsteen triplex *for identifying nucleic acid sequence* (emphasis added), as stated on page 8, lines 2-3, of the Office Action. The except reads as follows:

> In reference to a triplex, the term ["perfectly matched"] means that the triplex consists of a perfectly matched duplex and a third strand in which every nucleotide undergoes Hoogsteen or reverse Hoogsteen association with a basepair of the perfectly matched duplex.

Clearly, this excerpt is a definition of the term "perfectly matched" as it related to triplexes. It has nothing to do with how triplexes might be used. In particular, it states nothing about identifying nucleic acid sequences with triplexes. Accordingly, Applicants submit that the rejection has no grounds and should be withdrawn.

In part 10. of the Office Action, the Examiner rejected claims 17-18 under 35 U.S.C. 103(a) as being unpatentable over Brenner ('675) in view of Brenner ('097) for essentially the same reasons as summarized above in the obviousness-type double patenting rejection based on '097 in view of '675.

Applicant respectfully disagrees with the rejection for the reasons set forth above. Namely, the Examiner has merely identified the elements of the invention in the cited references, but has not provided any reasoning based on the disclosures for why one with ordinary skill in the art would be led to combine the elements to arrive at Applicants' invention. Applicants submit that the Examiner is merely using hindsight to reconstruct Applicants' invention from elements that are purportedly disclosed in the cited references. Accordingly, the rejection should be withdrawn.

In part 11. of the Office Action, the Examiner rejected composition claims 24-29 under 35 U.S.C. 103(a) as being unpatentable over Brenner '097. The Examiner argues that '097 discloses oligonucleotide tags of the same structure as called for in the instant invention and that '097 "indicates that the use of oligonucleotide tags is for identifying or sorting nucleic acid" sequences. Therefore, it would have been prima facie obvious to

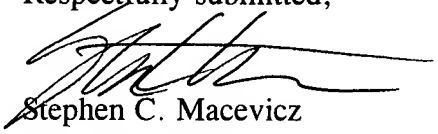
construct the encoded adaptors of the present invention.

Applicants respectfully disagree, particularly in view of the amendments. '097 nowhere teaches or suggests the use of oligonucleotide tags in combination with an adaptor. '097 only directly teaches the making and using of oligonucleotide tags to *sort* polynucleotides onto solid phase supports (making tags: col. 6, line 33, to col. 9, line 21; and using tags: col. 9, line 24, to col. 16, line 52; and the examples). In every case, oligonucleotide tags are attached to polynucleotides of unknown sequence and are used to specifically hybridize the resulting conjugates to tag complements attached to a solid phase support. In the instant invention, an oligonucleotide tag forms a part of an encoded adaptor and is used to specifically hybridize a labeled tag complement from solution in order to identify one or more nucleotides in the protruding strand of the encoded adaptor. Thus, the function of the tags and tag complements is quite different and, therefore, one with ordinary skill in the art would not be led to use oligonucleotide tags of '097 as labeling means. Accordingly, Applicants respectfully request that the rejection be withdrawn.

In view of the above, Applicants submit that the claims as written fully satisfy the requirements of Title 35 of the U.S. Code, and respectfully request that the rejections thereunder be withdrawn and the claims be allowed.

If any additional time extensions are required, such time extensions are hereby requested. If any additional fees not submitted with this response are required, please take such fees from deposit account **12-2491**.

Respectfully submitted,



Stephen C. Macevicz
Reg. No. 30,285
Attorney for Applicants

Telephone: (510) 670-9365
Enclosures:

Information Disclosure Statement with references.
Petition for Time Extension.
Fee Authorization.